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FGD1 Promote Ferroptosis in Model of CCl4-induced LF Through NRF2 by PTEN Signalling Pathway

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KEYWORDS LF. Ferroptosis. FGD1. Nrf2. PTEN

ABSTRACT This study was to explore the feasibility of FGD1 in a model of Liver fibrosis (LF) to evaluate its mechanism. Serum FGD1 mRNA expression was up-regulated in patients with LF and had a positive correlation with serum α-SMA, Collagen I, and E-cadherin mRNA levels. FGD1 levels in liver tissue were increased. *In vitro* model of LF, FGD1 promoted ferroptosis of hepatic fibroblasts and reduced cell growth, in CCl4-induced LF mice. FGD1 induced PTEN/Nrf2 signalling pathway. Sh-FGD1 prevented LF in mice. *In vitro* model of LF, the inhibition of PTEN reduced the effects of FGD1 on hepatic fibroblasts. PTEN reduced LF in LF mice by Sh-FGD1. Taken together, FGD1 induces the PTEN/Nrf2 pathway to promote ferroptosis of hepatic fibroblasts in LF and provide molecular insight into the mechanisms by which the FGD1 regulates ferroptosis in LF.

INTRODUCTION

Liver fibrosis (LF) is a manifestation of repair imbalance resulting from chronic injury (Ding et al. 2023a; Djouina et al. 2023). There is today general agreement that LF begins with the recruitment of inflammatory immune cells caused by cellular injury. LF continuous accumulation of extracellular matrix caused by various pathogenic factors, including hepatoviral infection, alcohol, metabolic disorders, autoimmune disorders, and hepatotoxic drugs.

The chronic inflammatory response of the liver is mainly mediated by liver macrophages. Macrophages have strong plasticity. Under pathological conditions, in addition to resident macrophages, macrophages from other sources are constantly recruited into tissues and differentiate into different phenotypes based on changes in the tissue microenvironment. Alpha-smooth muscle actin (á-SMA) myofibroblasts produce a lot of collagen and other extracellular matrix (ECM) molecules, and secrete pro-inflammatory cytokines to exacerbate subsequent immune cell infiltration and promote fibrosis (Ding et al. 2023b; Lurie et al. 2015). Research has shown that after removing the root causes of various chronic liver diseases, some patients can achieve fibrosis reversal. However, spontaneous reversal usually occurs relatively slowly or with a low incidence, making it difficult to avoid life-threatening complications in a timely manner, especially late stage fibrosis.

Further investigations have revealed that during this process, activated hepatic stellate cells (HSCs) undergo cellular apoptosis or senescence, resulting in the reversal of LF. HSCs are a key driving factor in experimental and human liver injury-induced fibrosis. Therefore, specifically targeting and clearing activated HSCs can prevent excessive ECM deposition and is regarded as a key measure for treating and preventing LF of LF. Carbon tetrachloride (CCl_4) has been widely used as an organic solvent, chemical raw material, and fabric dry cleaning agent, increasing the opportunities for occupational and non-occupational contact, and posing a potential threat to the population year by year (Roehlen et al. 2020). CCl_4 poisoning is mainly characterised by central anaesthesia symptoms and liver and kidney damage (Zhang et al. 2021). Toxicological studies found that CCl_4 can cause liver cell necrosis, steatosis, fibrosis, and carcinogenesis (Zhao et al. 2021).

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Lysosome-mediated regulated cell death (RCD) also important to LF except lipid metabolism (Kong et al. 2019). Hepatocyte apoptosis is mainly triggered by both intrinsic and extrinsic factors activating Caspase-7 and Caspase-3, cause to protein cleavage, nuclear fragmentation, and cell death. The inflammation caused by RCD is much less than that caused by necrosis. Faced with metabolic imbalance, inflammation, and other factors inducing mitochondrial dysfunction and endoplasmic reticulum stress (ERS), hepatocytes spontaneously exhibit regulatory mechanisms for RCD, including autophagy, pyroptosis, and ferroptosis (Liu et al. 2022). These mechanisms aim to remove intracellular debris, modulate hepatocellular paracrine secretion to reduce inflammation and regulate iron metabolism abnormalities that inhibit lipid oxidation, thereby protecting normal hepatocyte function. Responsing to ERS induced by hepatitis hepatitis virus, signal pathways involving tumour necrosis factor (TNF) and p53, can death receptors attract cytotoxic T cells,regulate cellular apoptosis, for targeted clearance of hepatocytes (Huang et al. 2022). Although cytotoxic T cells attempt to minimise local inflammation through controlled apoptosis, prolonged high-level antigen exposure can lead to T cell exhaustion, which is an important factor contributing to fibrosis (Luo et al. 2022).

Ferroptosis is worth considering whether inducing ferroptosis in HSCs or LF can alleviate. The mechanisms of Ferroptosis are mainly summarised as iron metabolism, glutathione peroxidase 4 (GPX4)/glutathione (GSH) (Yang et al. 2022). Inhibiting the GSH/GPX4 system can lead to cell membrane peroxidation damage and ferroptosis (Guo et al. 2023). GPX4 is the only known GPXs isoenzyme that can reduce lipid peroxides to alcohols, and inhibit the occurrence of ferroptosis (Lang et al. 2023). GSH is an important cofactor for GPX4 to exert antioxidant activity (Yuan et al. 2022). Lack of GSH will lead to GPX4 inactivation, and its synthesis is controlled by the rate limit of cysteine (Lu et al. 2023). Knocking out GPX4-related genes or using GPX4 inhibitors such as RSL3 will induce cell ferroptosis, which is related to lipid peroxidation caused by intracellular GPX4 inactivation (Gong et al. 2023).

The nuclear factor E2 related factor 2 (Nrf2)/GPX4 is an important regulatory pathway for ferroptosis (Shi et al. 2023d). Research has found that the protein arginine methyltransferase 4 can promote ferroptosis by inhibiting the Nrf2/GPX4 pathway, thereby exacerbating doxorubicin-induced cardiomyopathy (Pan et al. 2023). In addition, research has confirmed that aerobic exercise can inhibit ferroptosis and promote myocardial antioxidant enzyme activity and improving LF by activating the Nrf2/GPX4 pathway (Shi et al. 2023c; Xiong et al. 2021). Nrf2 forms a dimer with its inhibitory protein Keap1 in the cytoplasm (Yang et al. 2023). As an important regulator of ferroptosis, GPX4 is regulated by Nrf2. Upon stimulation, Nrf2 dissociates from its inhibitory protein Keap1, GPX4, and heme oxygenase 1, thereby exhibiting antioxidant activity (Ruan et al. 2020).

FGD1 promoted the synthesis of fibronectin and collagen in ECM, and it can also induce fibrosis and activate cancer matrix, making it a key growth factor in tumour EMT (Wu et al. 2020; Hou et al. 2003). Secondly, cells form proteolytic active protrusions on the ECM matrix, commonly referred to as invasive pseudopodia, which can lead to local degradation of ECM and promote tumour metastasis (Zeng et al. 2020).

Objective

This study explored the feasibility of FGD1 in the model of LF to evaluate its mechanism.

MATERIAL AND METHODS

Patients with LF

This study was approved by the Ethics Committee of our hospital. All the serum samples from normal volunteers and patients with LF were snap-frozen in liquid nitrogen and stored at -80°C.

Cell Culture and Transfection

Using Lipofectamine 2000, FGD1 plasmid or si-FGD1 plasmid were transfected into LX-2 cells (human hepatic stellate cell line). After 4 hours of transfection, LX-2 cells were given with the recommended (5 μM) of TGF-β1 for 48 hours.

***In Vivo* Model**

Every 3 days, old C57/BL6 male mice were intraperitoneally injected with 0.6 μl/g of CCL_4 (CCL_4: olive oil = 1:3), administered for 8 weeks continu-

ously. The liver and serum were harvested and the mice were sacrificed, one day after the last administration of CCL_4.

Quantitative Polymerase Chain Reaction (qPCR)

According to the Prime-ScriptTM RT detection kit, qPCR was performed with the ABI Prism 7500 sequence detection system. Relative levels of the sample mRNA expression were expressed as $2^{-\Delta\Delta Ct}$ and calculated.

Immunofluorescent Staining

After blocking with 5 percent BSA for 1 hour, LX-2 cells were incubated with PTEN (1:500, Cell Signalling Technology, Inc.) and FGD1 (1:100, Abcam) at 4ÚC overnight. Cells were incubated with goat anti-rabbit IgG-cFL 488 or anti-rabbit IgG-cFL 555 antibody (1:100) for 2 hours at room temperature washed with PBS for 15 minutes, and stained with DAPI. The images of cells were obtained by using a Zeiss Axioplan 2 fluorescence microscope (Carl Zeiss AG, Oberkochen, Germany).

Proliferation Assay and EDU Staining

After culturing at the indicated time, using CellTiter-GloR Luminescent Cell Viability Assay (Promega, Madison, WI, U.S.A.) , the cellular proliferation was detected. Cells were fixed with 4 percent formaldehyde for 30 minutes, EdU (10 mM, Beyotime) was added to each well using a fluorescent microscope (Olympus).

Western Blot

The membranes were incubated with primary antibodies: FGD1 (ab251655, 1:1000, Abcam), GPX4 (ab252833, 1:1000, Abcam), Nrf2 (12721, 1:1000, Cell Signalling Technology, Inc.), PTEN (9559, 1:1000, Cell Signalling Technology, Inc.), and β-Actin (4970, 1:5000, Cell Signalling Technology, Inc.), followed by incubation with secondary antibodies (1:5000, servicebio).

Statistical Analyses

Statistical analysis was performed by GraphPad Prism 6 using one-way analysis of variance (ANOVA) or Student's t-test. $p < 0.05$ was considered statistically significant.

RESULTS

Expression of FGD1 Levels in Patients with LF

Firstly, this paper mainly studies the levels of FGD1 in a model of LF. Serum FGD1 mRNA expression in patients with LF was up-regulated, comparison with the normal volunteers ($p<0.01$; Fig. 1A). Serum FGD1 mRNA expression was positively correlated with α-SMA ($p=0.0008$), Collagen I ($p=0.0010$) and E-cadherin ($p=0.0002$) mRNA expression levels in LF patients, and AUC = 0.9444 (Fig. 1B-1E). In LF mice model, FGD1 mRNA and protein expression levels in liver tissue were increased in comparison with the sham operation group ($p<0.01$; Fig. 1F,1G).

Serum FGD1 mRNA expression (A), FGD1 mRNA expression was positively correlated with Collagen I (B), α-SMA (C), E-cadherin (D) mRNA expression in patients, and Sensitivity (E), FGD1 mRNA and protein expression (F and G). $^{\#\#}p<0.01$ vs sham operation groups or normal volunteers

FGD1 Promoted Hepatic Fibroblasts in Vitro Model of LF

The researchers tested the function of FGD1 on hepatic fibroblasts *in vitro* model of CCl_4-induced LF. si-FGD1 plasmid reduced FGD1 mRNA expression of hepatic fibrocytes, FGD1 plasmid increased FGD1 mRNA expression *in vitro* model of LF ($p<0.01$; Fig. 2A). FGD1 increased the mRNA expression levels of COL1A1, α-SMA, Collagen I and E-cadherin *in vitro* model of LF ($p<0.01$; Fig. 2B-2E). Si-FGD1 decreased the mRNA expression levels of COL 1A1, α-SMA, Collagen I, and E-cadherin *in vitro* model of LF ($p<0.01$; Fig. 2F-2I).

FGD1/col1A1/α-SMA/Collagen I/E-cadherin mRNA expression (A/B/C/D/E), COL 1A1/α-SMA/Collagen I/E-cadherin mRNA expression (F/G/H/I) *in vitro* model of LF. $^{**}p<0.01$ vs the negative or si-NC group.

FGD1 Reduced Cell Growth *in Vitro* Model of LF

The study examined the effects of FGD1 on cell growth in vitro model of LF. FGD1 reduced cell growth and EDU-positive cells, and decreased

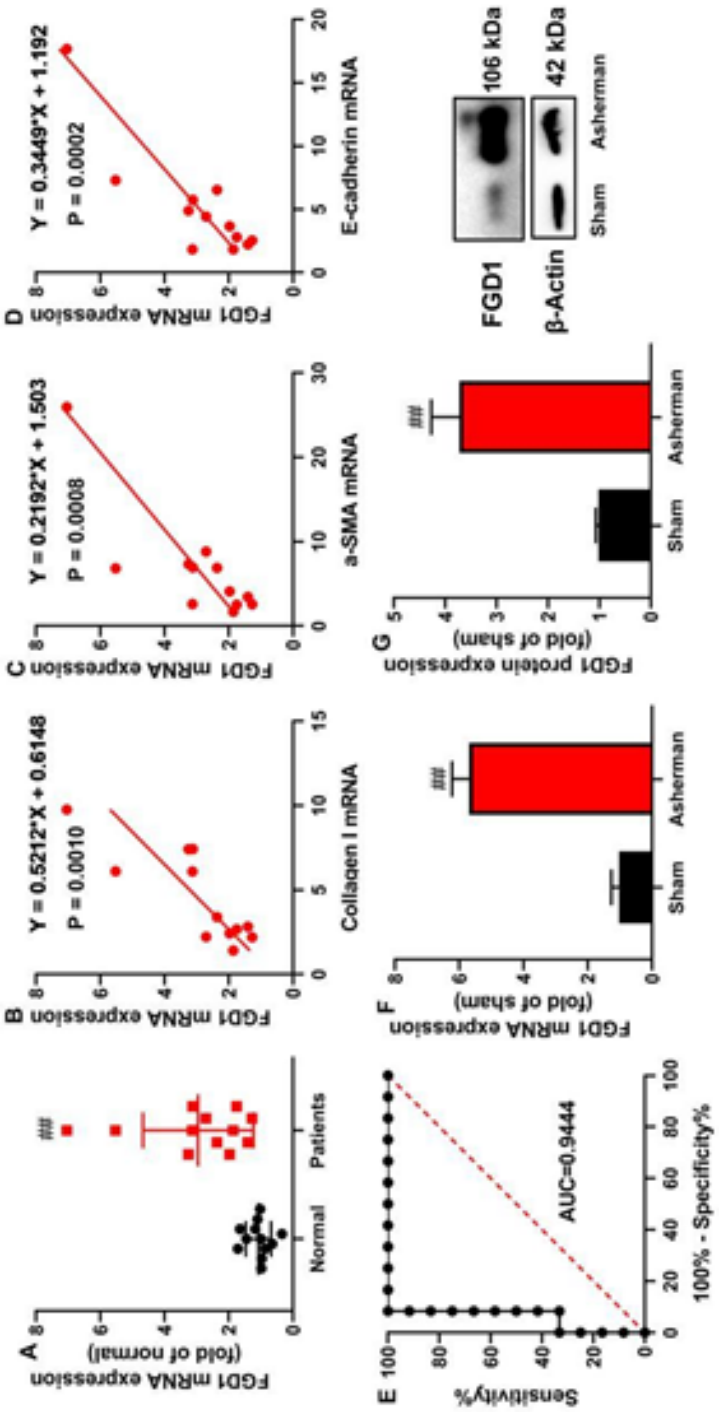

Fig. 1. Expression of FGD1 levels in patients with LF
Serum FGD1 mRNA expression (A), FGD1 mRNA expression was positively correlated with Collagen I (B), á-SMA (C), E-cadherin (D) mRNA expression in patients, and Sensitivity (E), FGD1 mRNA and protein expression (F and G). ##$p<0.01$ vs sham operation groups or normal volunteers

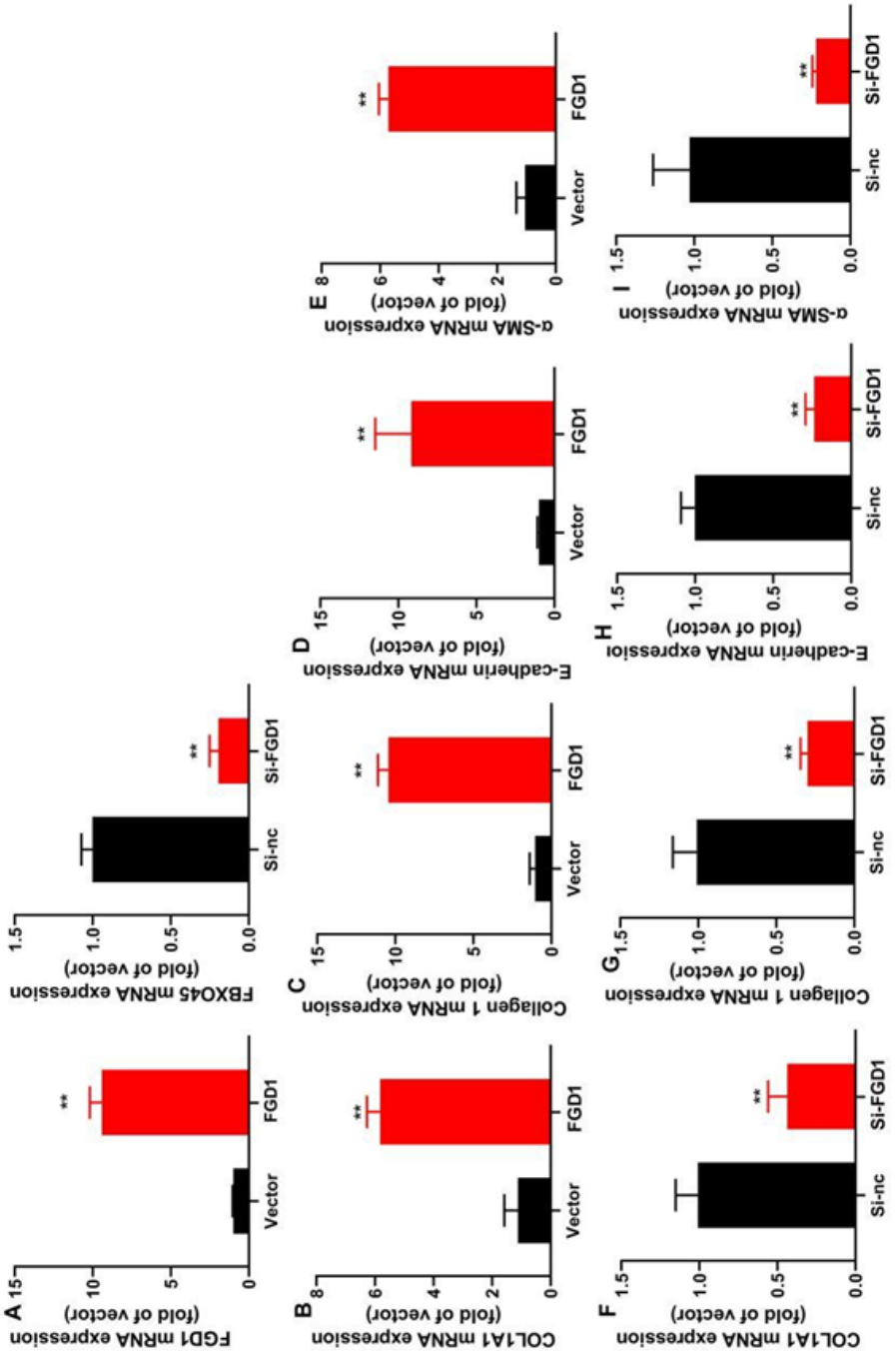

Fig. 2. FGD1 promoted hepatic fibroblasts in vitro model of LF
FGD1/col1A1/á-SMA/Collagen I/E-cadherin mRNA expression (A/B/C/D/E), COL 1A1/á-SMA/Collagen I/E-cadherin mRNA expression (F/G/H/I) *in vitro* model of LF. $^{**}p<0.01$ vs the negative or si-NC group

migration rate in *vitro model* of LF (p<0.01; Fig. 3A-3C). Si-FGD1 increased cell growth and EDU-positive cells and promoted migration rate *in vitro* model of LF (p<0.01; Fig. 3D-3F).

FGD1 Promoted Ferroptosis of Hepatic Fibroblasts *in Vitro* Model of LF

The researchers sought to understand the effects of FGD1 on the ferroptosis of Hepatic Fibroblasts in a model of LF. FGD1 increased PI-positive cells and LDH activity levels and promoted iron concentration levels *in vitro* model (p<0.01; Fig. 4A-4C). Si-FGD1 down-regulation reduced PI-positive cells and LDH activity levels and inhibited iron concentration levels of hepatic fibroblasts *in vitro* model of LF (p<0.01; Fig. 4A-4C). FGD1 reduced GSH activity level and suppressed GPX4 protein expression levels of hepatic fibroblasts *in vitro* model of LF (p<0.01; Fig. 4D-4E). Si-FGD1 increased GSH activity level, and induced GPX4 protein expression levels of hepatic fibroblasts in vitro model of LF (p<0.01; Fig. 4D-4E).

FGD1 over-expression reduced JC-1 levels and MPT and promoted mitochondrial damage in a model of LF (p<0.01; Fig. 4F-4H). Si-FGD1 promoted JC-1 levels and MPT and reduced mitochondrial damage of hepatic fibroblasts in vitro model of LF (p<0.01; Fig. 4F-4H).

FGD1 Induced PTEN/ Nrf2 Signalling Pathway

Serum FGD1 mRNA expression was negatively correlated with serum PTEN (p=0.0102) and Nrf2 mRNA (p=0.0058) expression levels in patients with LF (Fig. 5A). FGD1 over-expression induced FGD1 and PTEN protein expression levels, and suppressed Nrf2 protein expression level *in vitro* model of LF (p<0.01; Fig. 5B). Si-FGD1 suppressed FGD1 and PTEN protein expression levels, and induced Nrf2 protein expression level *in vitro* model of LF (p<0.01; Fig. 5C). Immunofluorescence showed that FGD1 over-expression increased FGD1 and PTEN expression of hepatic fibroblasts *in vitro* model of LF (Fig. 5D). FGD1 protein interlinked with PTEN protein (Fig. 5E). FGD1 reduced PTEN Ubiquitination, and si-FGD1 increased PTEN Ubiquitination in vitro model of LF (Fig. 5F).

Sh-FGD1 Prevented LF in Mice Model of LF

The study determined the effect of Sh-FGD1 on LF in the mice model of LF. Sh-FGD1 virus prevented LF, reduced ALT, AST, HA, and Hyp levels, and suppressed COL1A1, Fibronectin, α-SMA, Collagen I, and E-cadherin mRNA expression levels in mice model of LF (p<0.01; Fig. 6A-6J). Then, the Sh-FGD1 virus suppressed PTEN expression and induced Nrf2 and Gpx4 protein expression levels in liver tissue of mice model of CCl_4-induced LF (p<0.01; Fig. 6K).

Inhibition of PTEN Reduced the Effects of FGD1 on LF in Vitro Model

More importantly, the researchers confirmed the mechanism of FGD1 on hepatic fibroblasts in vitro model by PTEN. PTEN inhibitor (100 nM of PTEN-IN-5) suppressed PTEN protein expression level, and induced GPX4 and Nrf2 protein expression levels *in vitro* model of LF by FGD1 (p<0.01; Fig. 7A). PTEN inhibitor increased cell growth, reduced hepatic fibroblasts and ferroptosis *in vitro* model of LF by FGD1 (p<0.01; Fig. 7B-7H, Fig. 8).

PTEN Reduced LF in Mice Model of LF by Sh-FGD1

Next, PTEN agonists suppressed GPX4 and Nrf2 protein expressions in liver tissue of mice model of LF by Sh-FGD1 virus (p<0.01; Fig. 9A). PTEN agonists increased LF and Liver injury in mice model of CCl_4-induced LF by Sh-FGD1 virus (p<0.01; Fig. 9B-9J).

DISCUSSION

LF is caused by various factors including viruses, alcohol, and autoimmune factors that cause inflammation and necrosis of liver cells, leading to activation and transformation, and a large amount of deposition in the intercellular matrix (Elbaset et al. 2023; Kisseleva and Brenner 2021). LF and early cirrhosis are reversible, but later progression to the late stage of cirrhosis is irreversible, and it may even develop into liver cancer (Tang et al. 2023; Caligiuri et al. 2021). LF is often associated with viral infections (such as HBV and HCV), NAFLD, non-alcoholic steatohepatitis (NASH), excessive alcohol intake, metabolic abnormalities, and bile stasis. Cirrhosis is an increasingly serious health problem in the world, with a global mortality rate of 2.4 percent in 2019. The incidence rate of NAFLD related liver failure and HCC is also on the rise, leading to NAFLD becoming the fastest growing

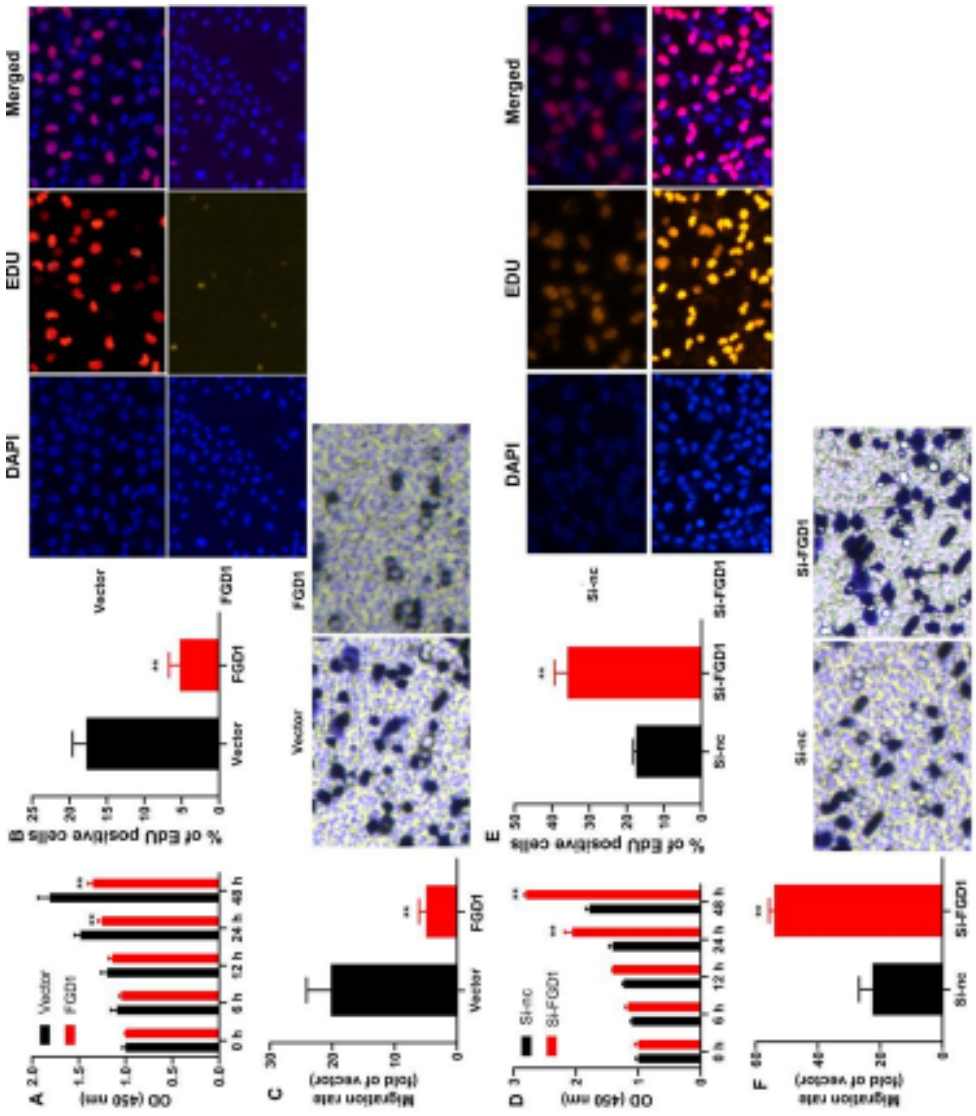

Fig. 3. FGD1 reduced cell growth in vitro model of LF
Cell growth (A), EDU positive cells (B), Migration rate (C) *in vitro* model of LF by FGD1; cell growth (D), EDU positive cells (E), migration rate (F) *in vitro* model of LF by si-FGD1. $^{**}p<0.01$ vs a negative or si-nc group.

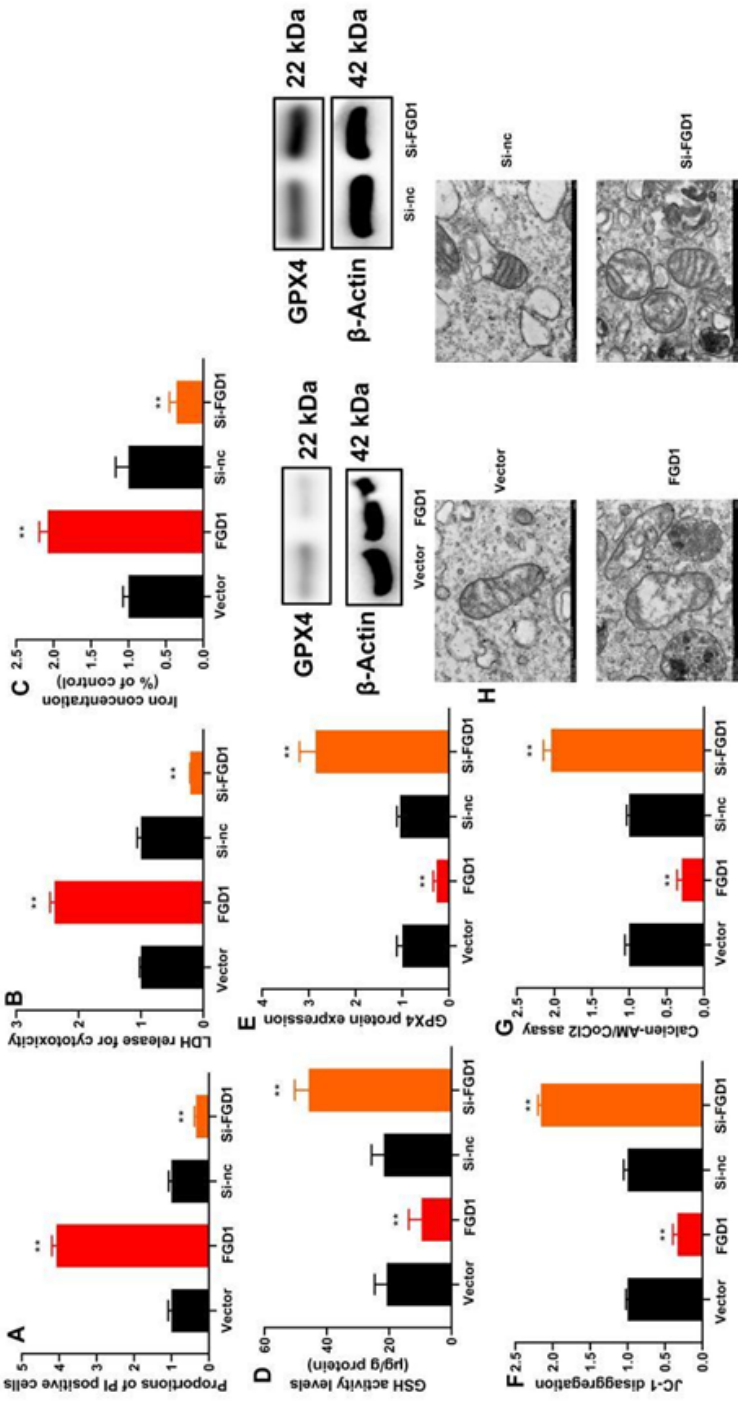

Fig. 4. FGD1 promoted ferroptosis of hepatic fibroblasts *in vitro* model of LF
PI-positive cells (A), LDH activity level (B), iron concentration levels (C), GSH level (D), GPX4 level (E), JC-1 levels (F), MPT (G), and mitochondrial damage (H). $^{**}p<0.01$ vs the negative or si-NC group

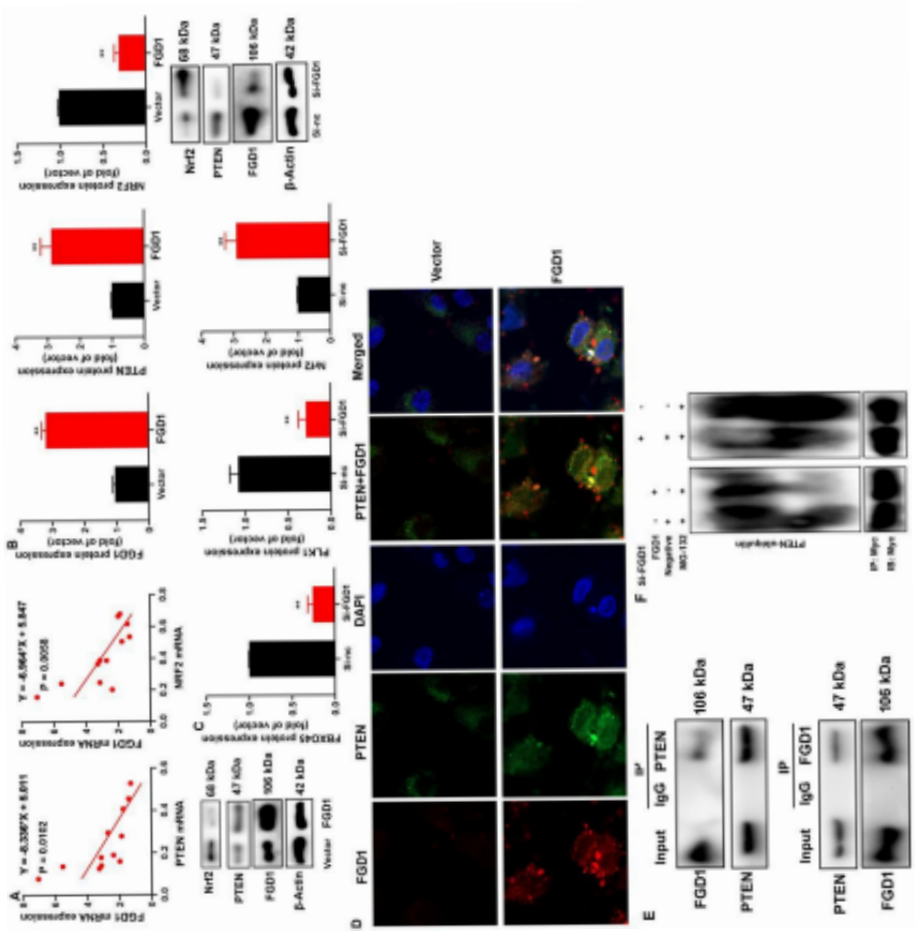

Fig. 5. FGD1 induced PTEN/Nrf2 signalling pathway
FGD1 mRNA expression was negative correlation with PTEN and Nrf2 mRNA expression (A), FGD1/PTEN/Nrf2 protein expression (B and C), Immunofluorescence (D), FGD1 protein interlinked with PTEN protein (E), PTEN Ubiquitination (F). $^{**}p<0.01$ vs the negative or si-NC group.

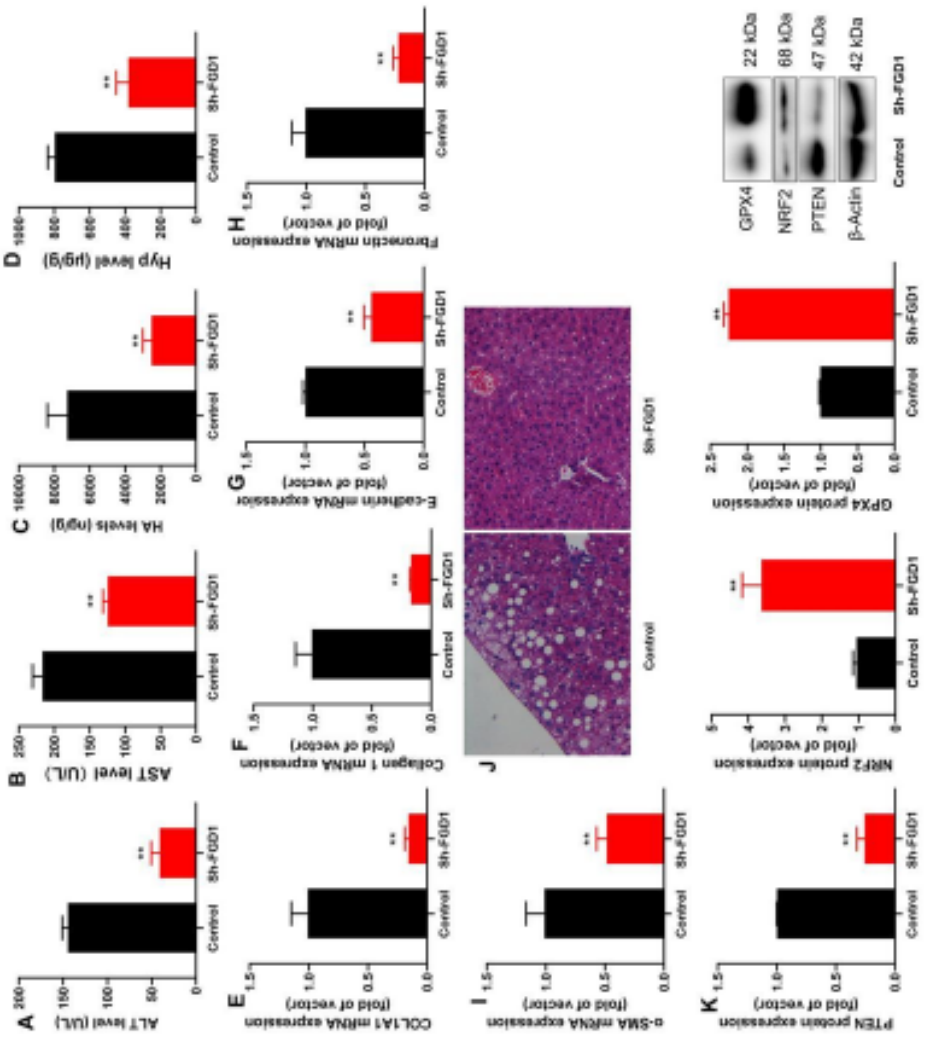

Fig. 6. Sh-FGD1 prevented LF in mice model of CCl_4-induced LF
ALT/AST/HA/Hyp levels (A, B, C, D), COL1A1, Fibronectin, á-SMA, Collagen I and E-cadherin mRNA expression (E, F, G, H, I), LF (J), PTEN/Nrf2/Gpx4 protein expressions (K). $^{**}p<0.01$ vs the Control group

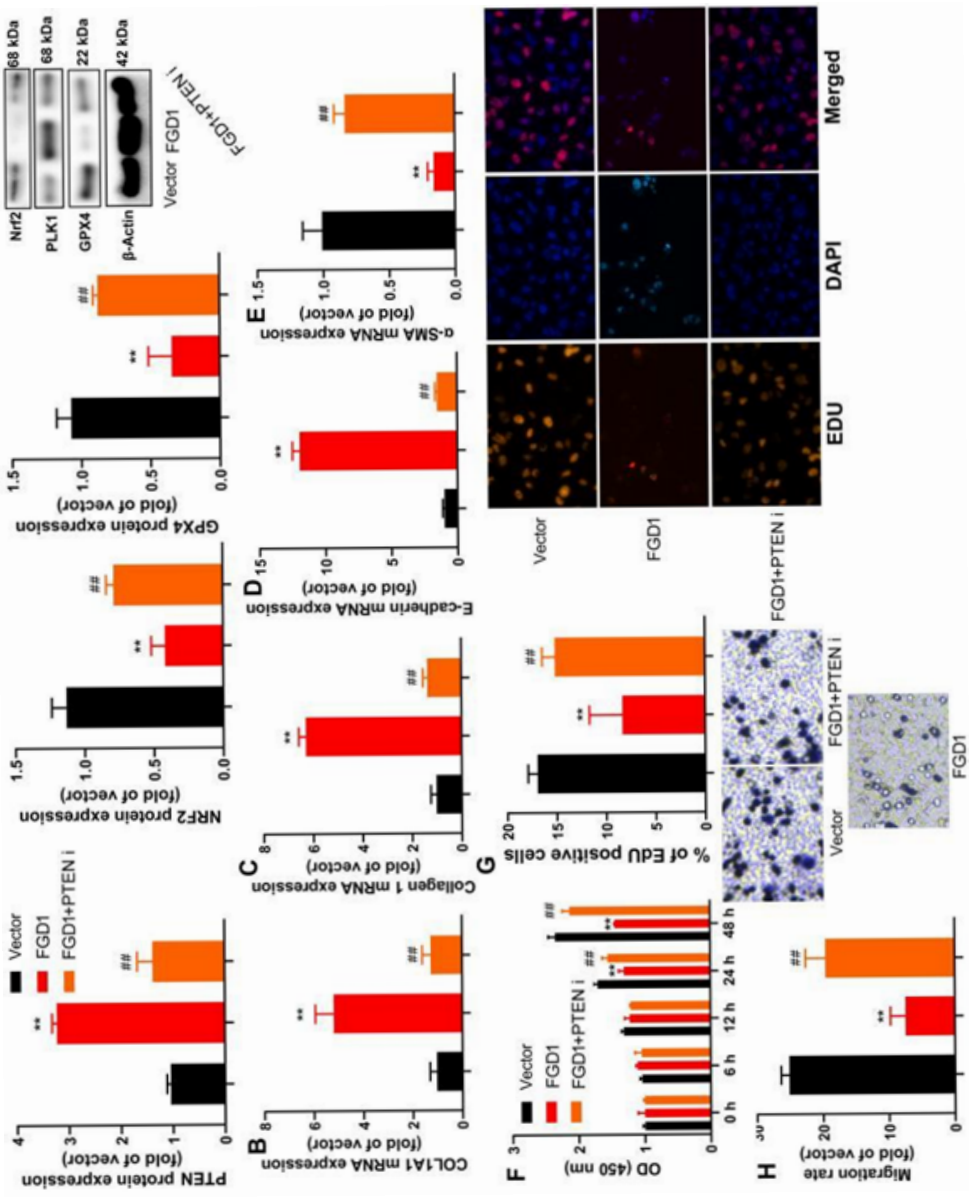

Fig. 7. Inhibition of PTEN reduced the effects of FGD1 on hepatic fibroblasts *in vitro* model of LF
PTEN/Nrf2/Gpx4 protein expression (A), col1A1/α-SMA/Collagen I/E-cadherin mRNA expression (B, C, D, E), cell growth (F), EDU positive cells (G), Migration rate (H). $^{\#\#}p<0.01$ vs the FGD1 group, $^{**}p<0.01$ vs Vector group

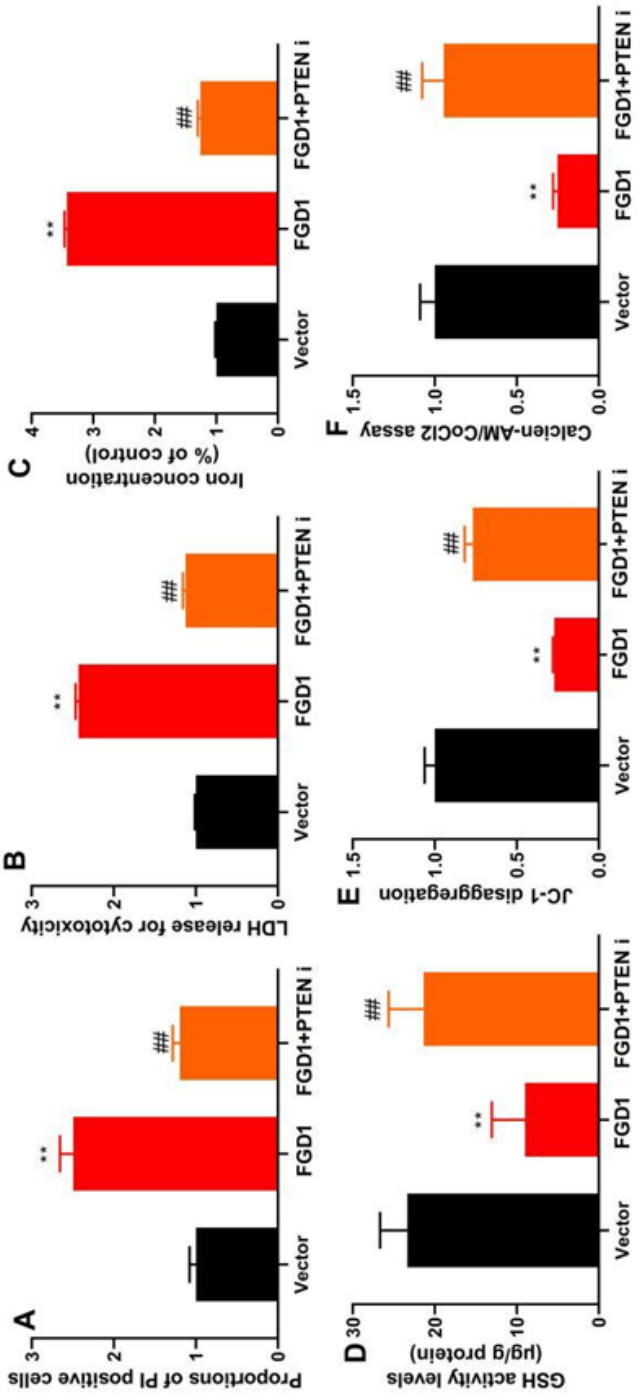

Fig. 8. Inhibition of PTEN reduced the effects of FGD1 on LF in vitro model by Ferroptosis
PI-positive cells (A), LDH activity level (B), iron concentration levels (C), GSH level (D), JC-1 levels (E), MPT (F). $^{**}p<0.01$ vs Vector group; $^{\#\#}p<0.01$ vs the FGD1 group

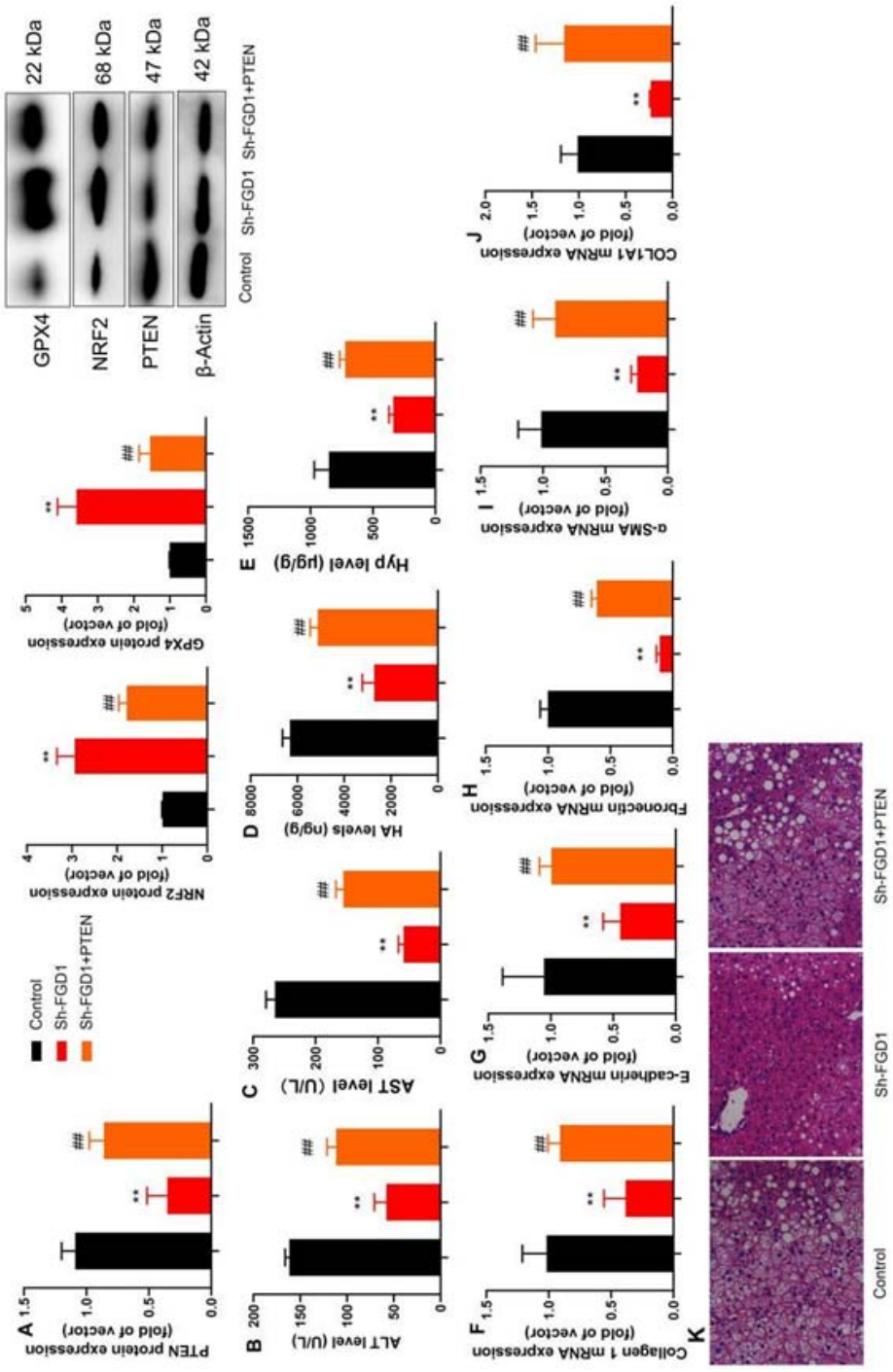

Fig. 9. PTEN reduced LF in mice model of LF by Sh-FGD1
PTEN/Nrf2/Gpx4 protein expression (A), ALT/AST/HA/Hyp levels (B, C, D, E), COL1A1, Fibronectin, α-SMA, Collagen I and E-cadherin mRNA expression (F, G, H, I, J), LF (K). $^{\#\#}p<0.01$ vs the FGD1 group
$^{**}p<0.01$ vs control group

factor in liver related mortality and incidence rate and the main factor in liver transplantation. Research has shown that the primary determinant of poor prognosis in NAFLD is the grading of fibrosis, rather than the histological characteristics of NASH. Even in the very early stages of fibrosis, the mortality rate increases slightly and linearly with the severity of fibrosis. Besides inflammation, each etiological factor has its own specific fibrogenic activation pathway in the process of LF development. HBV induces immune-suppressive cells accelerating LF through immune cascade reactions (Elbaset et al. 2023; Kisseleva and Brenner 2021). After exposure to various etiological factors, the body initially experiences the accumulation of immune cells and the activation of inflammation (Elbaset et al. 2023; Kisseleva and Brenner 2021). Based on existing research findings, the author summarises the pathogenic mechanisms as immune-inflammatory activation, cellular transformation, lipid engulfment, and regulatory cell death. These three factors contribute independently and interactively to the pathogenesis without a specific sequence, collectively leading to the occurrence and progression of fibrosis. Therefore, it is urgent to continuously seek effective treatment methods to block or even reverse LF. This study showed that serum FGD1 mRNA expression was up-regulated in patients with LF or mice model of LF. Zeng et al. (2020) reveal that FGD1 possesses oncogenic properties in hepatocellular carcinoma. FGD1 exerts a regulatory role in the model.

LF caused by multiple pathogenic factors leading to cytokine secretion and internal environmental disorders during long-term liver injury, resulting in excessive repair of the liver and the appearance of fibrous scars (Tang et al. 2023; Naume et al. 2023). The extracellular matrix is the material basis for the formation of LF (Li et al. 2023). In this study, FGD1 promoted hepatic fibroblasts, and reduced cell growth in vitro model of LF. Sh-FGD1 prevented LF in mice models. Cai et al. (2020) identified that FGD1 is one DNA methylation biomarker for hepatocellular carcinoma. So, FGD1 participated in LF in a mice model of CCl_4-induced LF.

Ferroptosis is closely associated with LF. Ferroptosis is characterised by iron-dependent redox imbalance, leading to membrane phospholipid peroxidation damage (Wu et al. 2021). Iron mediated cell death was first proposed in 2012, which is a regulatory cell death mode centered on iron. Therefore, inhibiting iron death has become one of the strategies for treating NAFLD fibrosis; On the other hand, promoting ferroptosis may also become a target for treating the fibrotic phase of NAFLD (Wu et al. 2021). Ferroptosis may become a new target for inhibiting LF (Pan et al. 2021). Moreover, abnormal elevation of iron ions and lipid peroxidation levels may have adverse effects on normal liver cells and the cellular environment within the liver. Unlike the clinical application of ferroptosis as a treatment for liver cancer, there have been no reports on clinical studies targeting ferroptosis. Currently, a critical question remains for how can one precisely and specifically induce ferroptosis in hepatic stellate cells at the individual level? This is the key to whether ferroptosis-based therapy for LF can be applied clinically in the future. FGD1 promoted ferroptosis of hepatic fibroblasts in vitro model of LF (Lang et al. 2023). Niu et al. (2021) suggested that FGD1 suppresses melanoma progression, and induces cell apoptosis. These data showed that the FGD1 gene promoted ferroptosis of hepatic fibroblasts in a model of LF (Chen et al. 2023; Shi et al. 2023b). Sh-FGD1 virus and induced Gpx4 protein expressions in liver tissue of mice model of CCl4-induced LF. Teles et al. (2012) found that FGD1 is involved in vesicular transport and cell death in the model of trauma. These data indicated that FGD1 participated in Ferroptosis of LF.

Nrf2 is the main regulatory factor of the antioxidant response, which can induce and participate in the regulation of iron concentration and intracellular iron ion metabolism. SLC7A11 and GPX4 are important downstream factors of Nrf2 (Li et al. 2023). The activation of Nrf2 can significantly improve LF (Yang et al. 2023; Shi et al. 2023a). This study showed that FGD1 induced PTEN/Nrf2 signalling pathways. So, by the inhibition of Nrf2 activity, FGD1 promoted ferroptosis of hepatic fibroblasts in a model of LF.

PTEN possesses diverse biological functions and is involved in cellular proliferation, survival, differentiation, and energy metabolism. Current research on the regulation of LF by PTEN mainly focuses on the PI3K/AKT pathway. It has also been found that PTEN can regulate macrophage M2 polarisation and epithelial-mesenchymal transition (EMT), although the specific mechanisms require further investigation. The expression of PTEN protein is significantly reduced in cancer tissue compared to adjacent tissues (Rojo et al.

2014). The lower the differentiation and malignancy of liver cancer tissue, the lower the expression of PTEN protein (Taguchi et al. 2014). This study determined that FGD1 up-regulation reduced PTEN Ubiquitination, and FGD1 down-regulation increased PTEN Ubiquitination in vitro model of LF. Wu et al. (2020) suggested that FGD1 promotes tumour progression in osteosarcoma by inhibiting PTEN activity. FGD1 gene induced PTEN activity to promote ferroptosis of hepatic fibroblasts in a model of LF by the inhibition of Nrf2 activity.

CONCLUSION

FGD1 gene promotes ferroptosis in model of LF through PTEN/Nrf2 signalling pathway by the inhibition of Nrf2 Ubiquitination.

RECOMMENDATIONS

The inhibition of FGD1 provides a rationale to enhance the efficacy of anti-ferroptosis treatment for hepatic fibroblasts. FGD1 might be a target for the treatment of LF or other liver diseases.

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